

BIOL 341
Bioinformatics project 1
Cloning of a Gene for Kanamycin Resistance into pUC19

Goal

The goal of this bioinformatics project is to plan a strategy for cloning a foreign gene into a plasmid. In order to do this you will need to use web based programs found on the NEB cutter server at <http://tools.neb.com/NEBcutter2/index.php>. This exercise is due in class on Tuesday September 19th. If you are having problems with the programs please come and see me in a timely manner so I can help you to have the project completed by the due date.

Introduction

The majority of specialized recombinant DNA molecules used in biotechnology have been constructed by a process known as sub-cloning. Sub-cloning involves the ligation (joining) of a previously cloned and purified DNA molecule into a vector molecule, in this case pUC19. The resulting recombinant molecule is then introduced into a cell where it can be propagated and expressed. Cells containing the recombinant molecule need to be screened in some manner as in many cases the vector can be re-ligated without the insert being present.

The Kan^r Gene:

Kanamycin is an aminoglycoside which interferes with translation by binding to a 70s prokaryotic ribosome. The drug interferes with translation by causing misreading of mRNA. The kanamycin resistance gene codes for a 3-aminoglycoside phosphotransferase which inactivates the drug by covalent modification. The modified drug cannot bind to the ribosomes.

Plasmid pGPS3:

This is the plasmid which contains the kan^r gene in addition to an ampicillin gene. The kanamycin gene is flanked by restriction sites that can be used to remove it for subcloning.

pUC18/19:

These plasmids have a multi cloning site (MCS) providing convenient restriction sites for insertion of foreign DNA.

Experimental Design

Using the NEB cutter site find the DNA sequence for plasmid pGPS3, you can cut and paste the nucleotide sequence or use the search option to find the correct plasmid in the database (this is the recommended approach). Use the default enzymes (the NEB enzymes) and plot restriction map(s) to allow identification of appropriate restriction sites (as discussed in class). Remember the goal is to cut and paste the kanamycin gene into the MCS of pUC 19 in the most efficient manner.

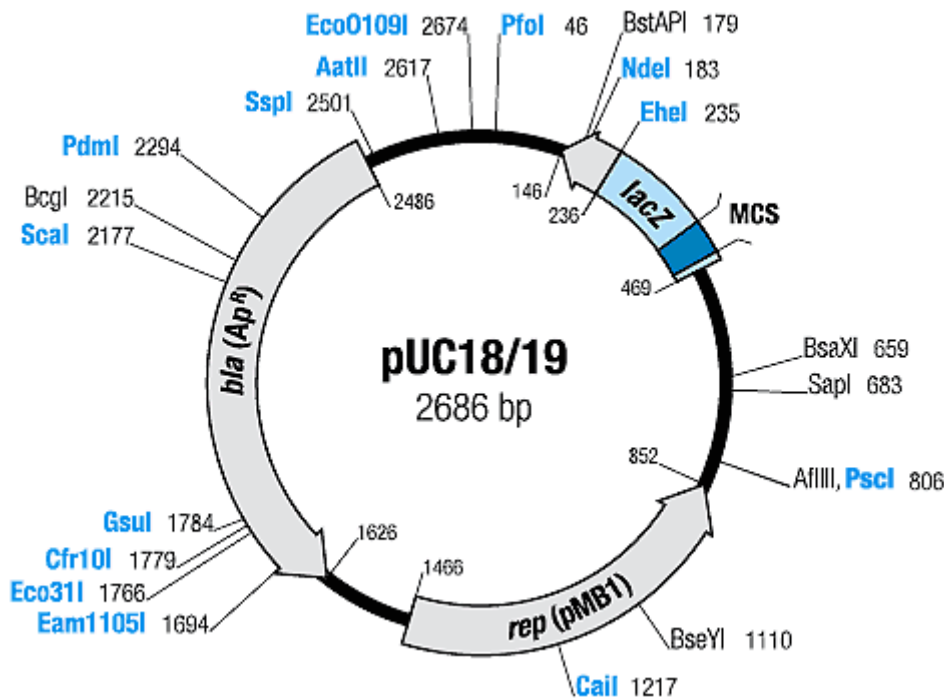
You should be able to identify several alternative enzymes that will achieve this goal. I know that we have EcoR1 and BamH1 enzymes available in the lab, I suspect we have several others too so be sure to identify alternatives.

The restriction maps for pUC18/19 are provided. you can use these or use the NEB cutter program to identify the restriction sites in the MCS that are available for insertion of the kan fragment.

The cloning strategy that you design should include information as to which enzymes will be used, the size of the kan fragment being excised and the size of the recombinant plasmid that results (pUC19 +

kan fragment). I expect to see a restriction map of pGPS3 showing the potential sites you will use to cleave out the kan fragment.

Choose one of your cloning strategies from above (ie one of the enzymes that you have identified) and use the NEB program to generate the recombinant molecule along with a restriction map for this new plasmid. For this step you will need to copy and paste the kan fragment into the pUC DNA sequence, it is easiest to do this in a word file, don't worry about the numbers they all go away once you paste back into the NEB cutter site, pasting correctly can be tricky so check that you did it right it may take a couple of attempts to get the sequences just right around the cut and paste sites, check by cutting the new plasmid with a test enzyme. I expect to see this map as part of your assignment. Be sure to save the sequences as they may be useful once you begin to do this experiment in the wet lab.



A complete project requires:

- Plasmid map of pGPS3 showing restriction sites.
- Identification of restriction sites that would be useful for a cloning experiment
- Plasmid map and identification of the appropriate restriction sites in pUC19
- The size of the kanamycin fragment being excised using the restriction enzyme that you chose to use.
- A plasmid map of the new recombinant plasmid that results from the ligation of the kan fragment into pUC19.
- A 'test cut' of this recombinant plasmid showing that the cloning was done accurately.